Determining Possible Fragments Based On Experimental Restrictions

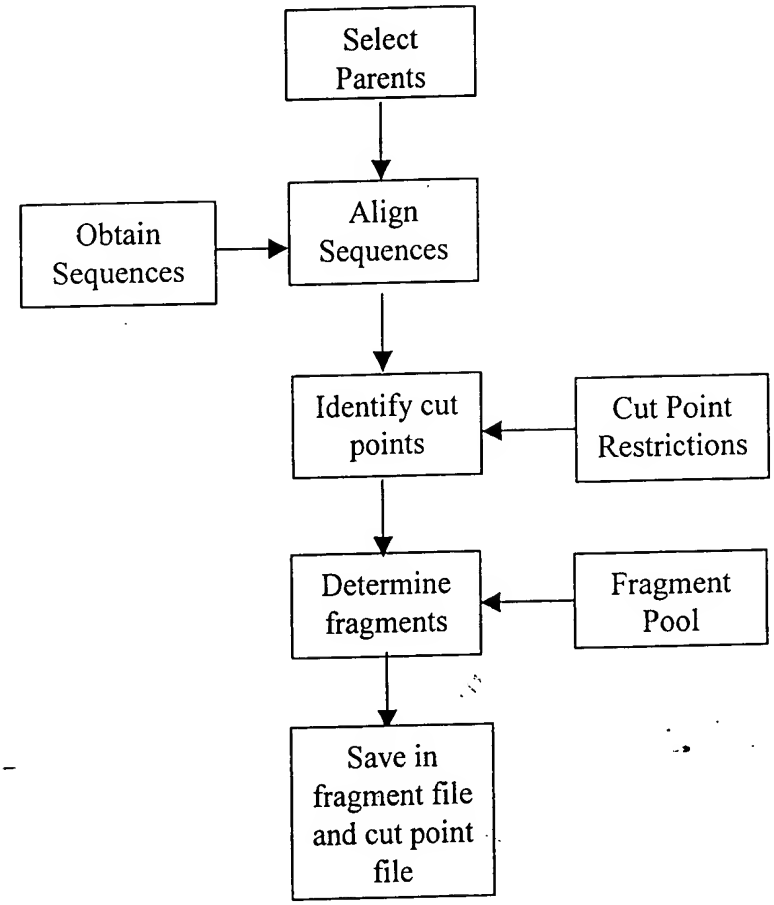


FIG. 1A

Determining the Schema Disruption Profile for a Structure

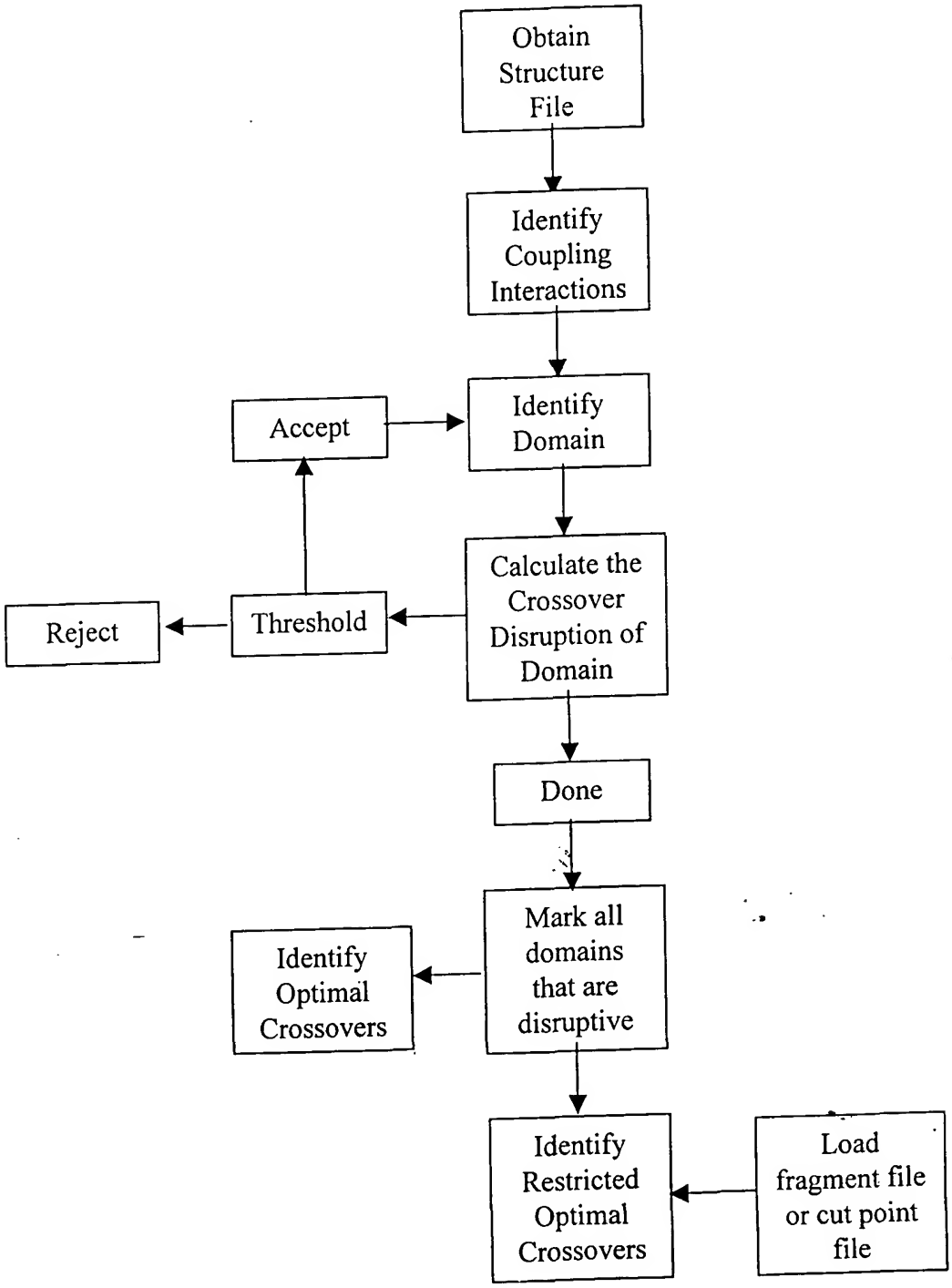


FIG. 1B

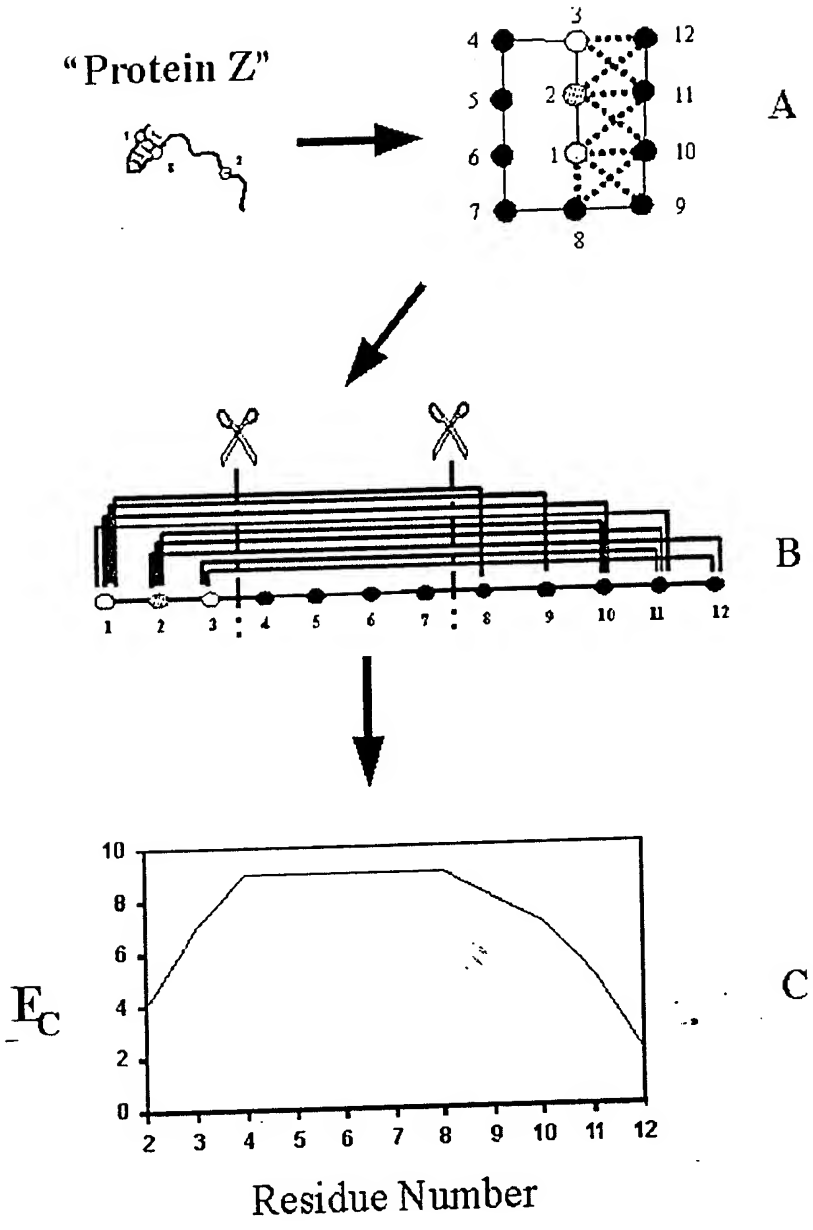


FIG. 2

```
1    1    T P V S E K Q L A E V V A N T I T P L M K A Q S V P G M A V A V I Y Q G K P H Y Y T F G K A D I A A
2    1    A K T E Q Q I A D I V N R T I T P L M Q E Q A I P G M A V A I I Y E G K P Y Y F T W G K A D I A N
3    1    T K L T E L Q V A T I V N N T L T P L L E K Q G I P G M A V A V F Y D G K P Q F F N Y G M A D I K A
4    1    Y A R G E A P L T A A V D G I I Q P M L K E Y R I P G M A V A V L K D G K A H Y F N Y G V A N R E S

1    51   N K P V T P Q T L F E L G S I S K T F T G V L G G D A I A R G E I S L D D A V T R Y W P Q L T G K Q
2    51   N H P V T Q Q T L F E L G S V S K T F N G V L G G D R I A R G E I K L S D P V T K Y W P E L T G K Q
3    51   G R P V T E N T L F E L G S V S K T F T G V A G E Y A M Q T G I M N L N D P V T E Y A P E L T G S Q
4    51   G Q R V S E Q T L F E I G S V S K T L T A T L G A Y A A V K G G F E L D D K V S Q H A P W L K G S A

1    101  W Q G I R M L D L A T Y T A G G L P L Q V P D E V T D N A S L L R F Y Q N W Q P Q W K P G T T R L Y
2    101  W R G I S L L H L A T Y T A G G L P L Q I P G D V T D K A E L L R F Y Q N W Q P Q W T P G A K R L Y
3    101  W K D V K M L H L A T Y T A G G L P L Q L P D S V T D Q K S L W Q Y Y Q Q W Q P Q W A P G V M R N Y
4    101  F D G V T M A E L A T Y S A G G L P L Q F P D E V D S N D K M R T Y Y R H W S P V Y P A G T H R Q Y

1    151  A N A S I G L F G A L A V K P S G M P Y E Q A M T T R V L K P L K L D H T W I N V P K A E E A H Y A
2    151  A N S S I G L F G A L A V K S S G M S Y E E A M T R R V L Q P L K L A H T W I T V P Q S E Q K N Y A
3    151  S N A S I G L F G A L A V K R S Q L T F E N Y M K E Y V F Q P L K L D H T F I T I P E S M Q S N Y A
4    151  S N P S I G L F G H L A A N S L G Q P F E Q L M S Q T L L P K L G L H H T Y I Q V P E S A I A N Y A

1    201  W G Y R D G K A V R V S P G M L D A Q A Y G V K T N V Q D M A N W V M A N M A P E N V A D A S L K Q
2    201  W G Y L E G K P V H V S P G Q L D A E A Y G V K S S V I D M A R W V Q A N M D A S H V Q E K T L Q Q
3    201  W G Y K D G Q P V R V T L G M L G E E A Y G V K S T S Q D M V R F M Q A N M D P E S L G N D K L K E
4    201  Y G Y K E D K P V R V T P G V L A A E A Y G I K T G S A D L L K F T E A N M G Y Q . . G D A A L K T

1    251  G I A L A Q S R Y W R I G S M Y Q G L G W E M L N W P V E A N T V V E G S D S K V A L A P L P V A E
2    251  G I E L A Q S R Y W R I G D M Y Q G L G W E M L N W P L K A D S I I N G S D S K V A L A A L P A V E
3    251  A I I A S Q S R Y F Q A G D M F Q G L G W E M Y S W P I N P Q G V I A D S G N D I A L K P R K V E A
4    249  R I A L T H T G F Y S V G D M T Q G L G W E S Y A Y P L T E Q A L L A G N S P A V S F Q A N P V T R

1    301  V N P P A P P V K A S W V H K T G S T G G F G S Y V A F I P E K Q I G I V M L A N T S Y P N P A R V
2    301  V N P P A P A V K A S W V H K T G S T G G F G S Y V A F V P E K N L G I V M L A N K S Y P N P A R V
3    301  L V P A Q P A V R A S W V H K T G A T N G F G A Y I V F I P E E K V G I V M L A N K N Y P N P V R V
4    299  F A V P K A M G E Q R L Y N K T G S T G G F G A Y V A F V P A R G I A I V M L A N R N Y P I E A R V

1    351  E A A Y H I L E A L Q
2    351  K A A W R I L E K L Q
3    351  Q A A Y D I L Q A L R
4    349  K A A H A I L S Q L A
```

1 Enterbacter cloacae P05364 (X03866)
2 Citrobacter freundii P05193 (X07274)
3 Yersinia enterocolitica P45460 (X63149)
4 Klebsiella pneumoniae Q48437 (X77455)

FIG. 3

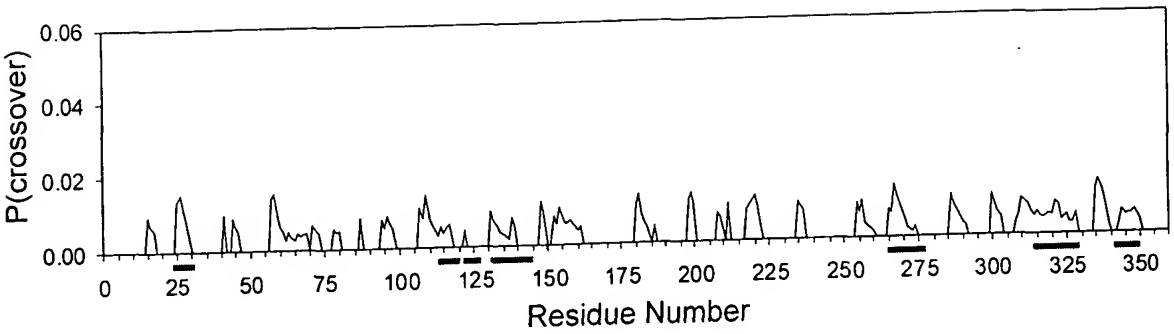


FIG. 4A

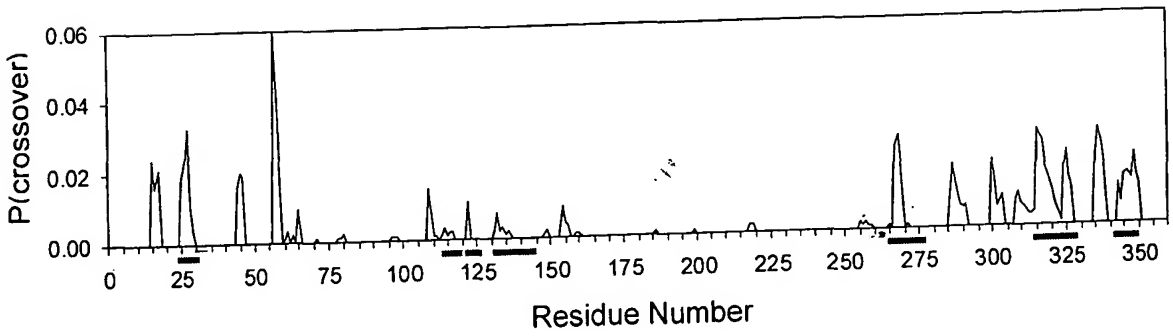


FIG. 4B

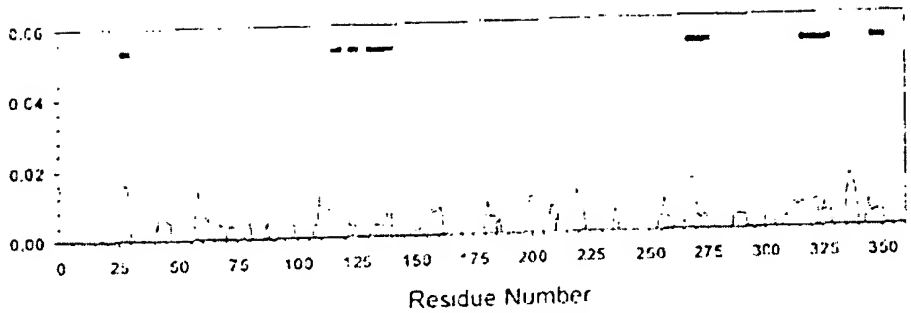

0.06
0.04
0.02
0.00
0 25 50 75 100 125 150 175 200 225 250 275 300 325 350
Residue Number

FIG. 4C

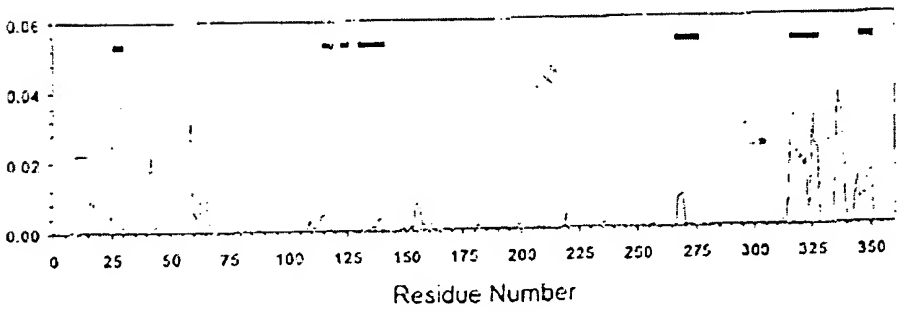

0.06
0.04
0.02
0.00
0 25 50 75 100 125 150 175 200 225 250 275 300 325 350
Residue Number

FIG. 4D

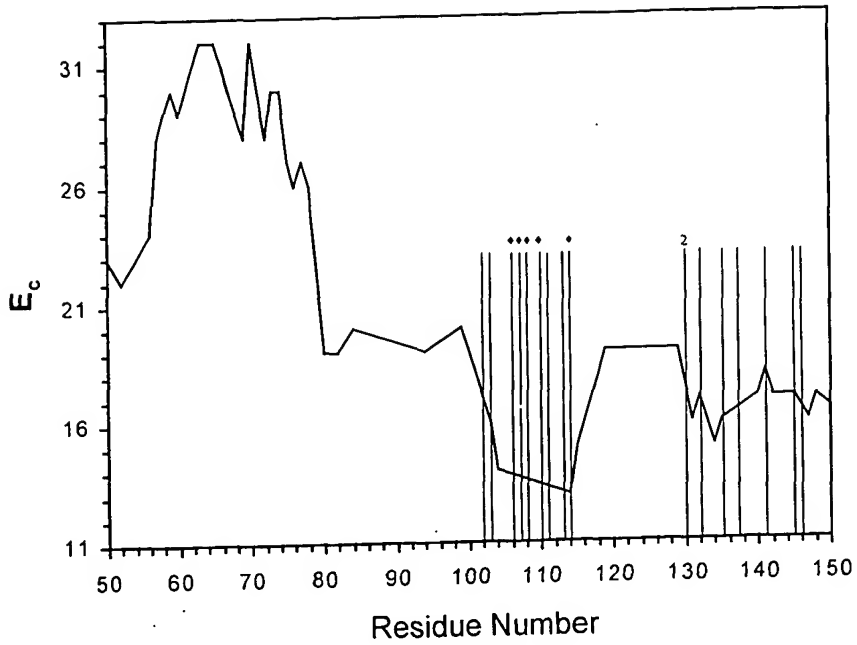

31
26
21
16
11
E_c
50
60
70
80
90
100
110
120
130
140
150
Residue Number

FIG. 5

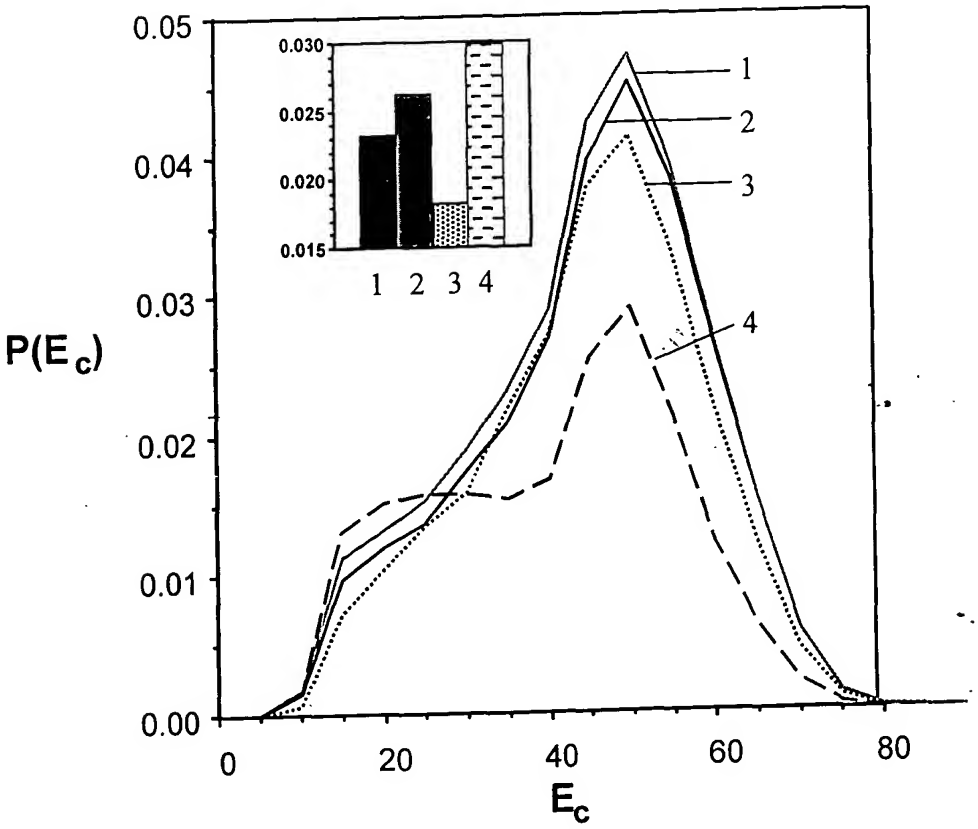
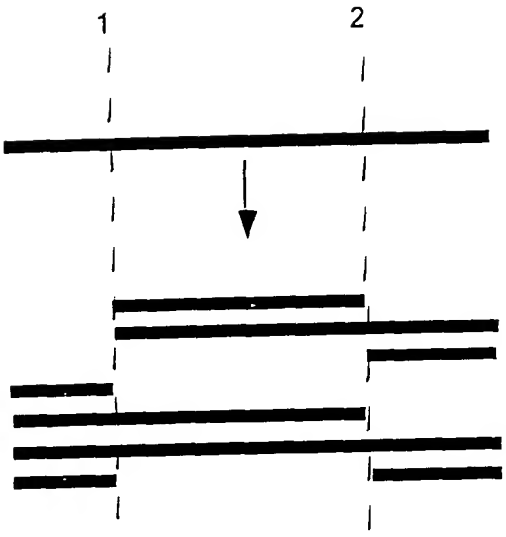

0.05
0.04
0.03
0.02
0.01
0.00
$P(E_c)$
0
20
40
60
80
E_c
0.030
0.025
0.020
0.015
1 2 3 4
1
2
3
4

FIG. 6

(A)

All possible recombinants prepared by crossover at positions 1 and 2



(B)

These can be prepared by assembly of synthetic fragments containing the crossover positions

Requires fragments (plus end primers):

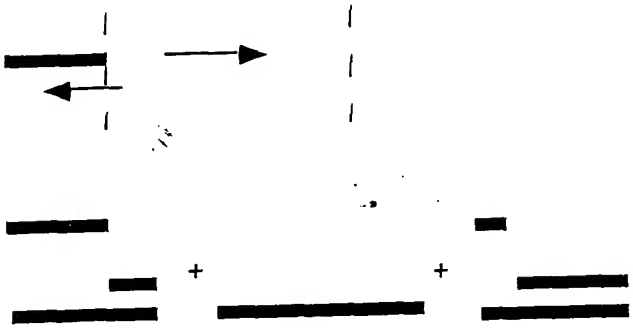


FIG. 7

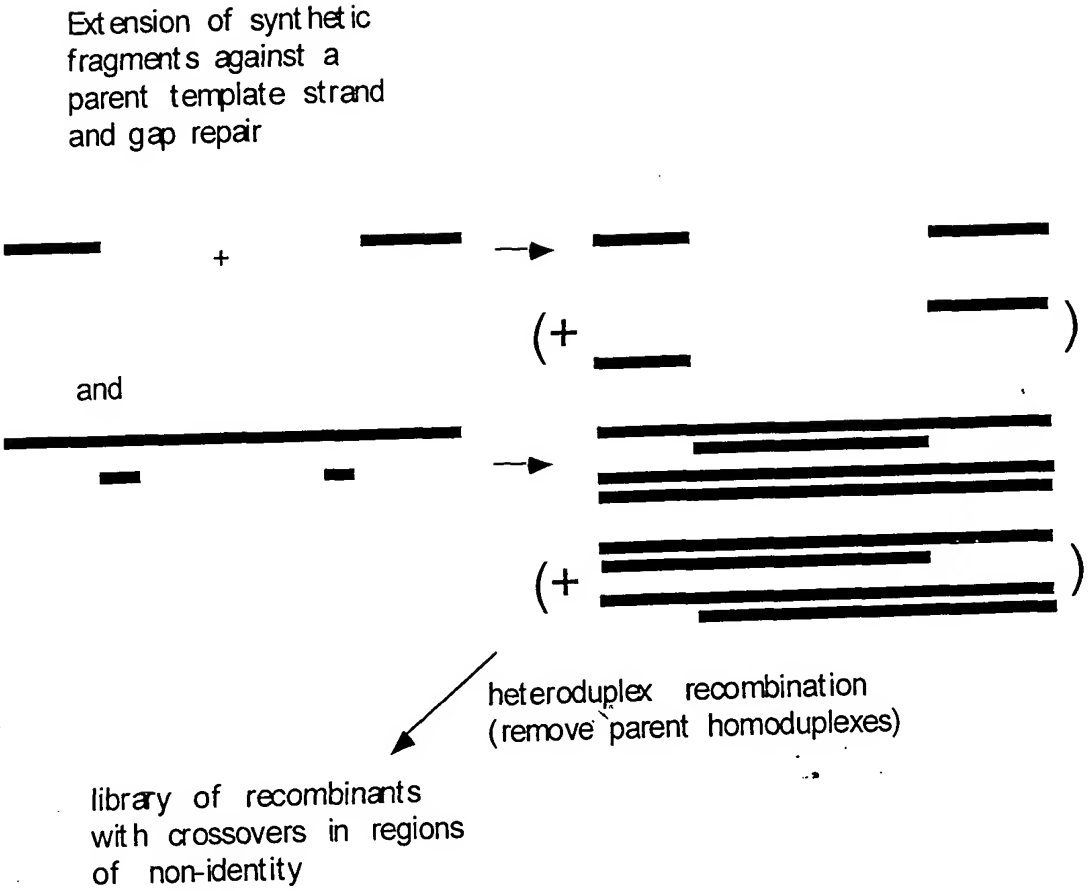
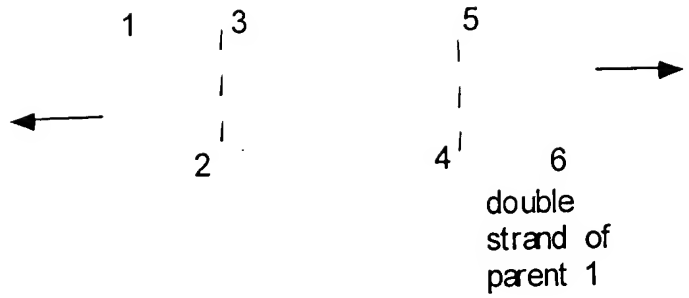


FIG. 8

(A)

Prepare the fragments by PCR with primers: perform reactions with primers 1+2, 3+4 and 5 +6,

and do same for other parent(s).



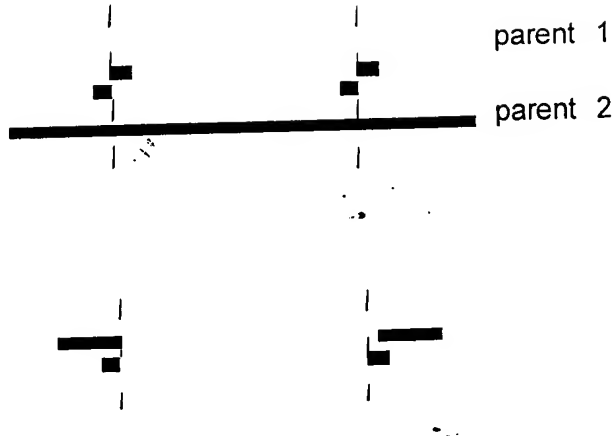
(B)

Reassemble fragments in a pool, by PCR with 1+ 6

FIG. 9

(A)

Prepare crossover primers designed to have crossovers at designated positions (2 primers for each position).



(B)

Fragment parent genes and PCR reassemble in the presence of the crossover primers to promote recombination at designated positions

FIG. 10

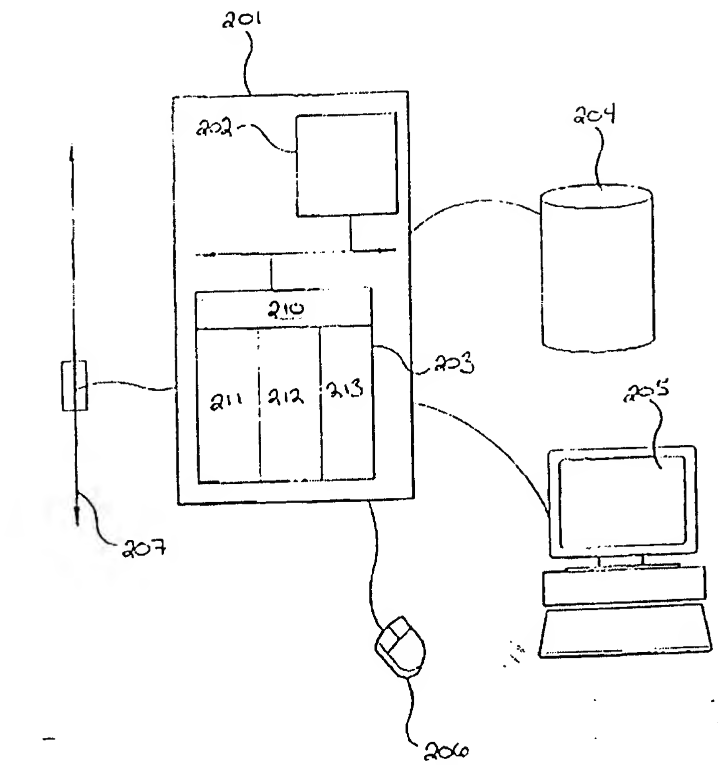
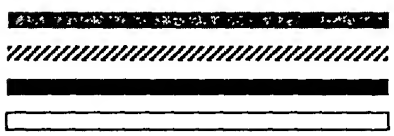


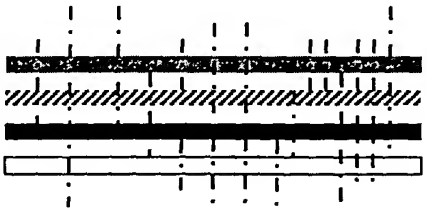
FIG. 11

Recombinant search algorithm

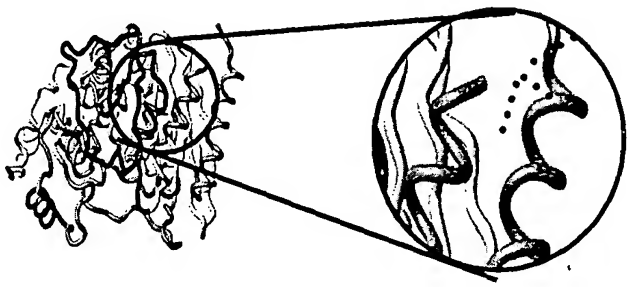
1. Align parent sequences with template structure



2. Determine all possible crossover points according to sequence identity algorithm

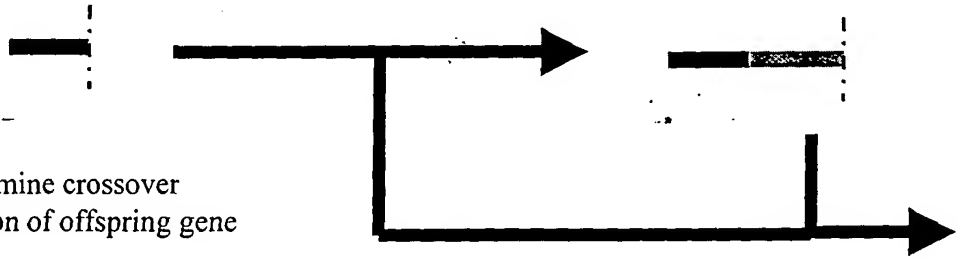


3. Calculate coupling matrix

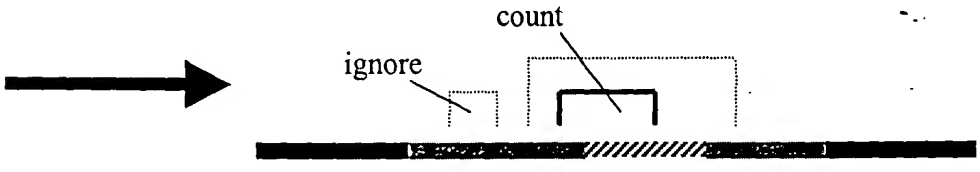


4. Pick start parent at random and copy to offspring until a possible cut point is reached

5. Pick random number, if less than *p*, copy random new parent until next cut point is reached.

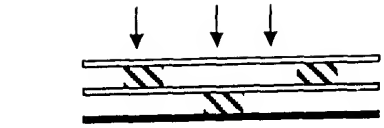


6. Determine crossover disruption of offspring gene

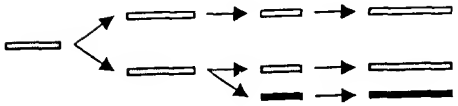


Offspring

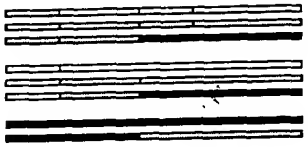
FIG. 12



(A)



(B)



(C)

FIG. 13

DIRECTED EVOLUTION ALGORITHM

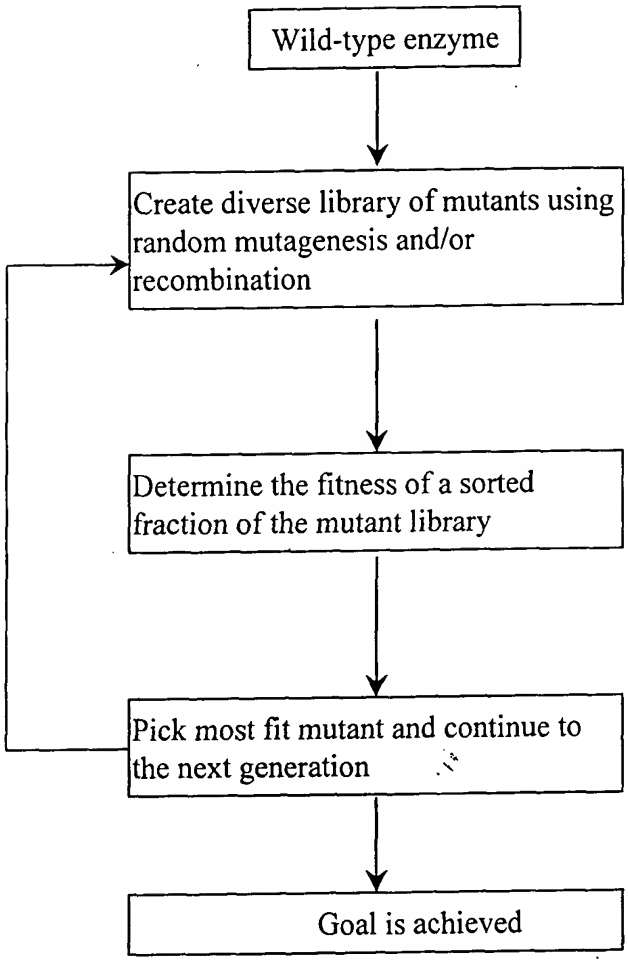


FIG. 14

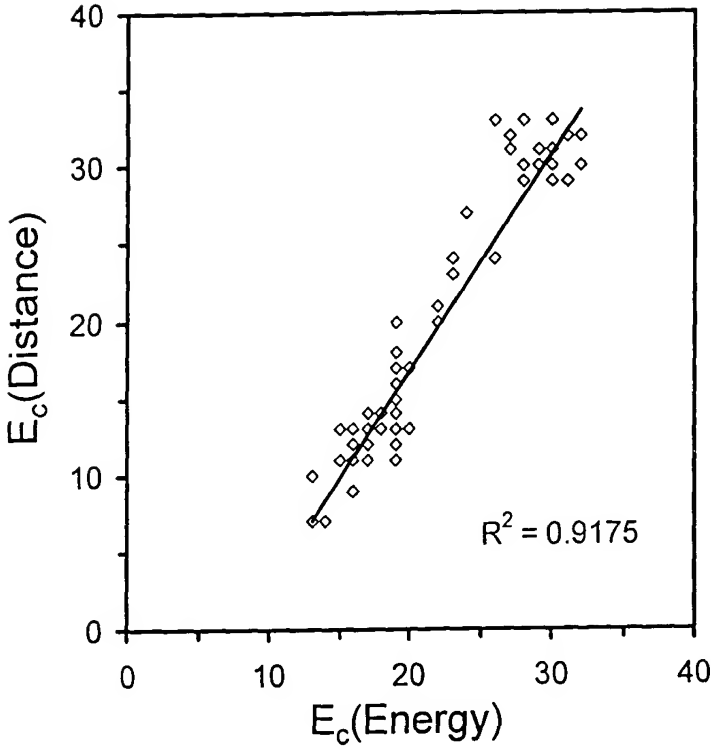


FIG. 15

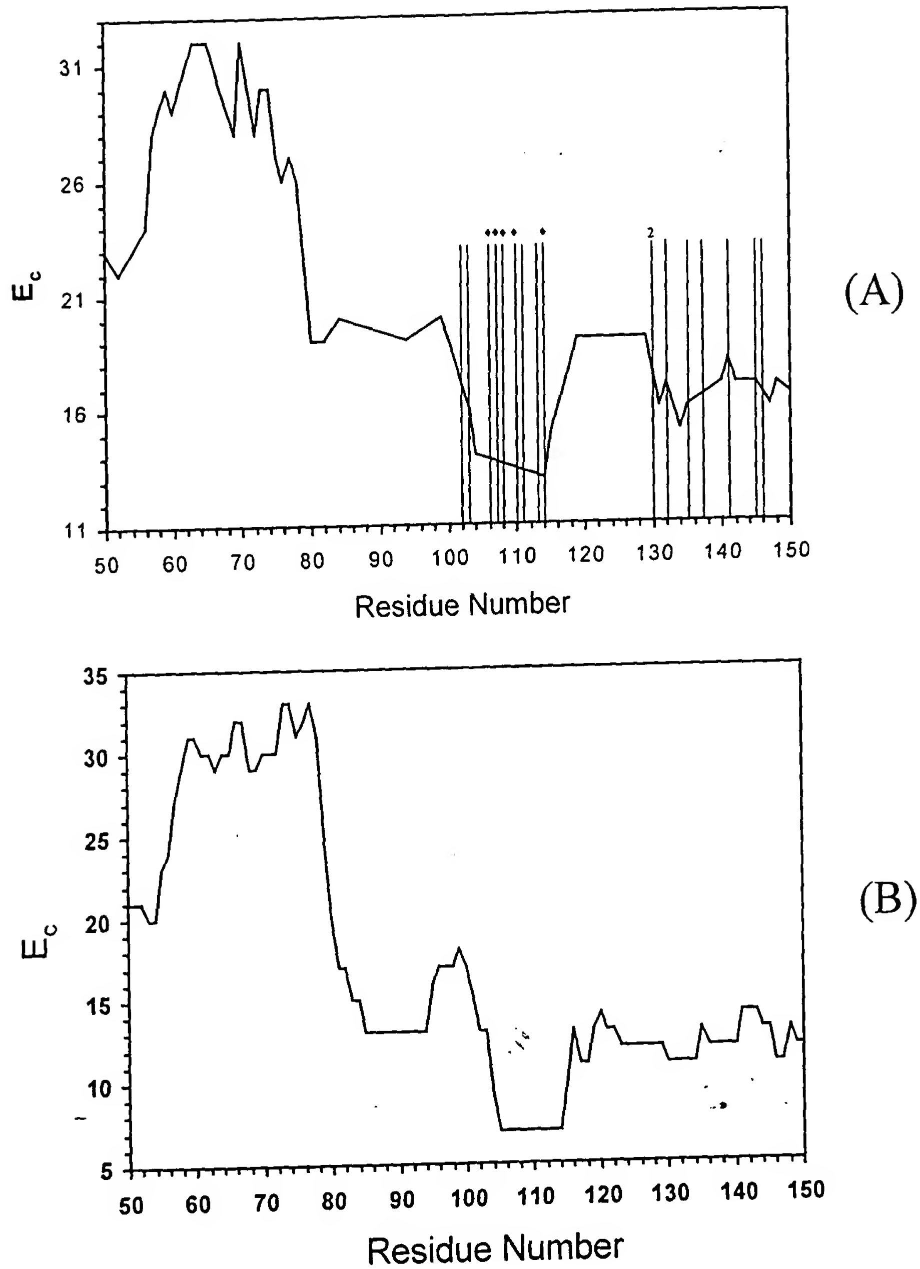


FIG. 16

(A)

Experimental Data:

	wt	wt-insert	1	2
Tm(dC)	52	55.2	n.d.	54.3
Tm(dC)	49.5	53.3	44.5	52.5
t1/2	12.1	2586	-	87.5
t1/2	53	138	4	308

(B)

Calculations:

	All schema		Fragments		Z-score	
	av	stdev	1	2	1	2
Ec	19.260	4.090	10.770	8.124	-2.076	-2.723
Ec*	0.006	0.002	0.014	0.005	4.838	-0.857

FIG. 17

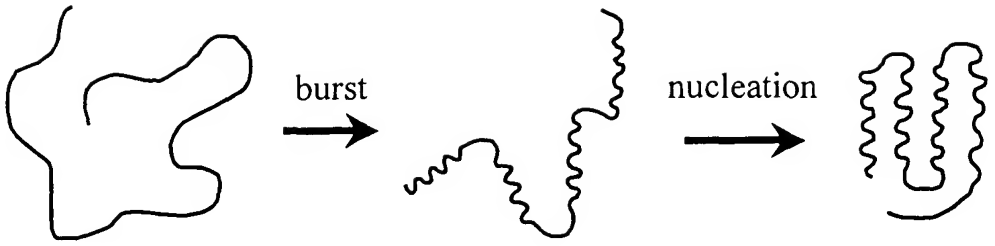


FIG. 18

The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, [redacted], folds an above average number of residues into a given sphere size, then it is compact.

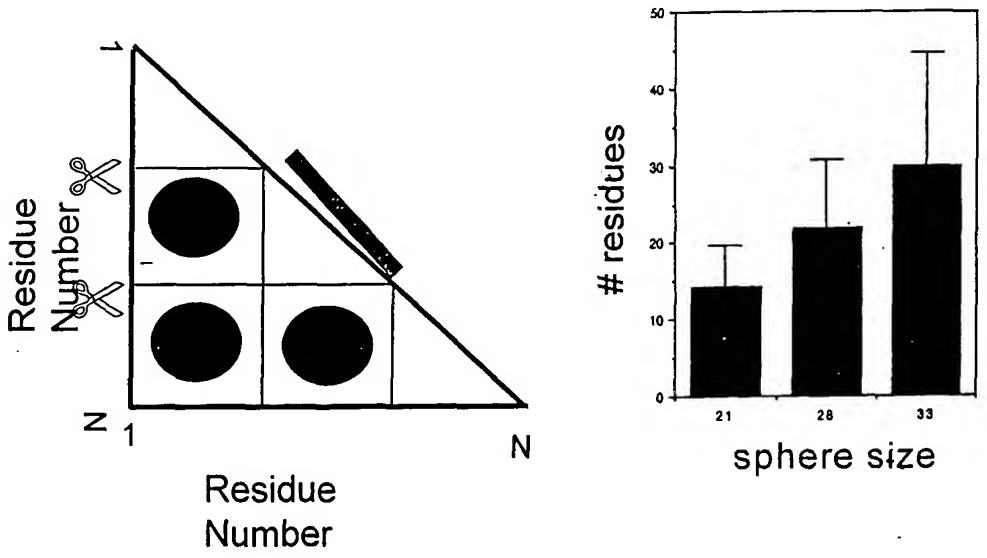
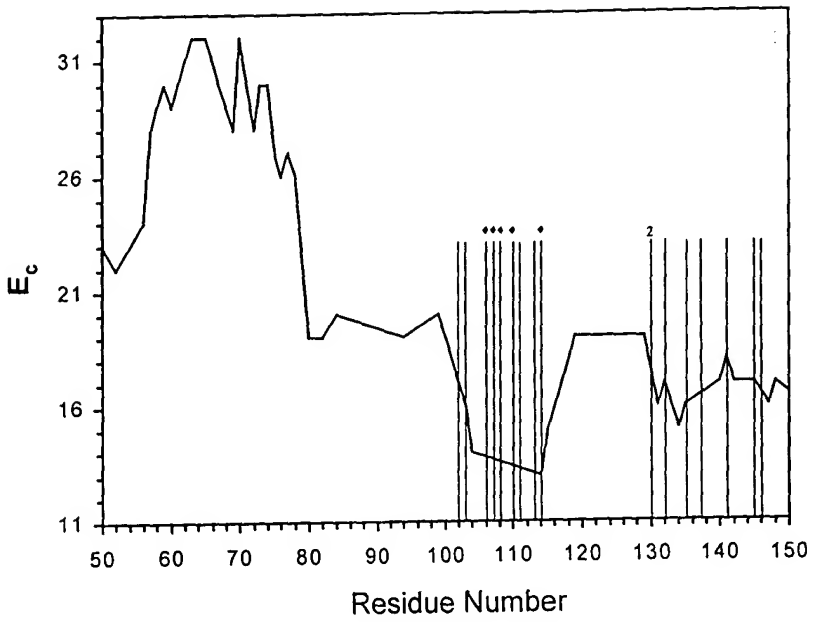
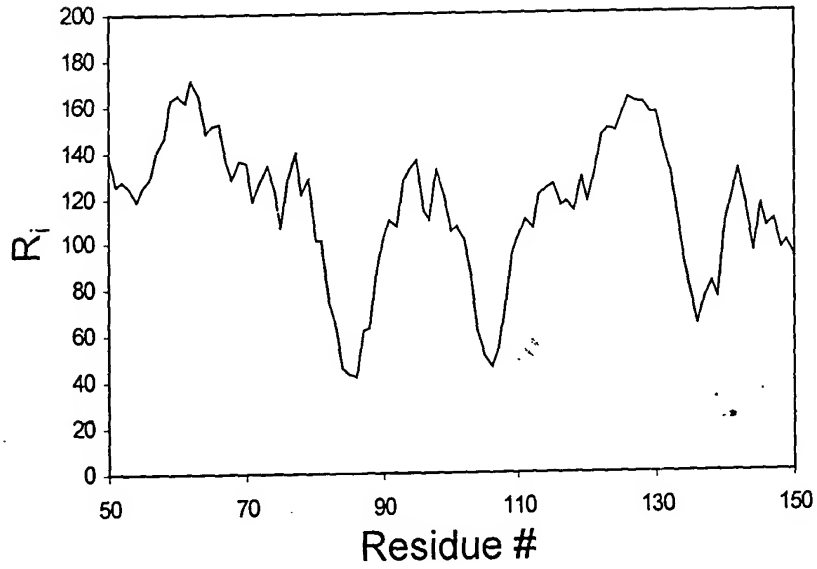


FIG. 19


31
26
21
16
11
E_c
50 60 70 80 90 100 110 120 130 140 150
Residue Number

(A)


200
180
160
140
120
100
80
60
40
20
0
R_i
50 70 90 110 130 150
Residue #

(B)

FIG. 20

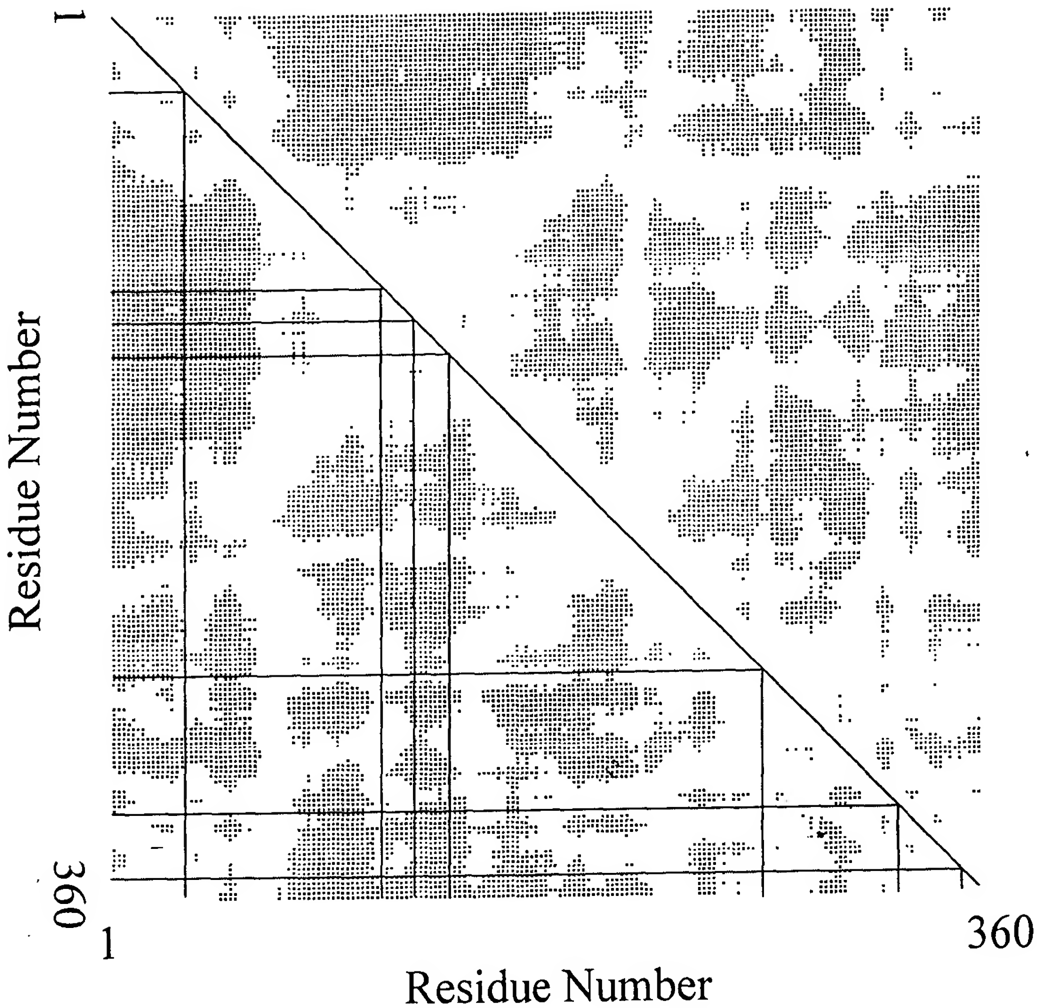


FIG. 21

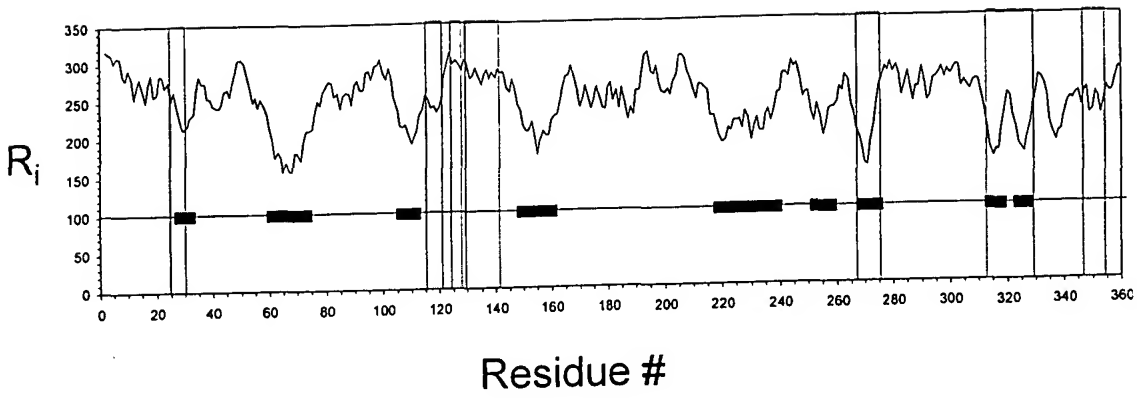
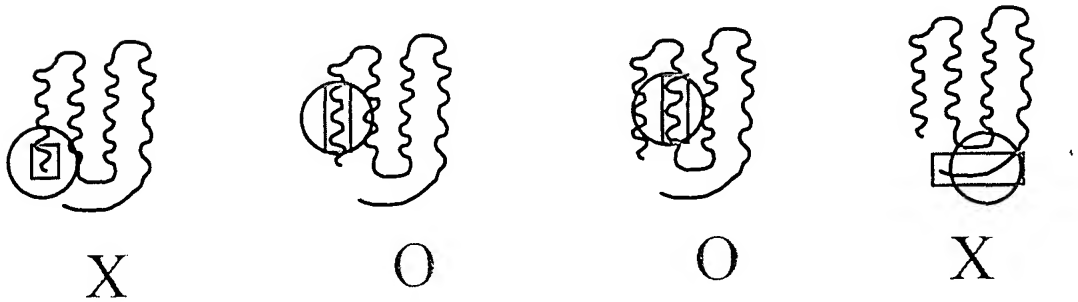
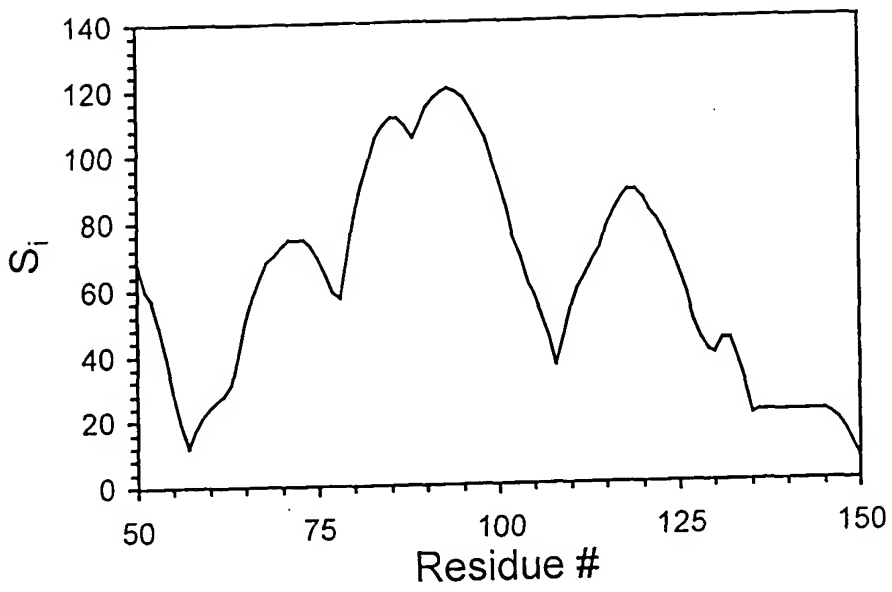


FIG. 22

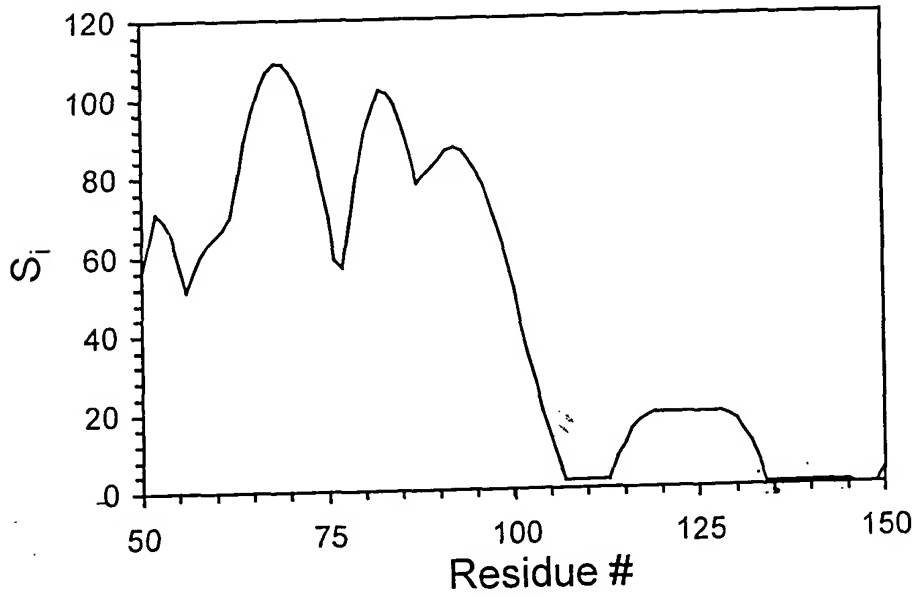


(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments at least the average number of residues for that sphere size or greater (e.g., >15 for 21 angstrom sphere); 3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; 4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

FIG. 23


140
120
100
80
60
40
20
0
S_i
50
75
100
125
150
Residue #

(A)


120
100
80
60
40
20
0
S_i
50
75
100
125
150
Residue #

(B)

FIG. 24

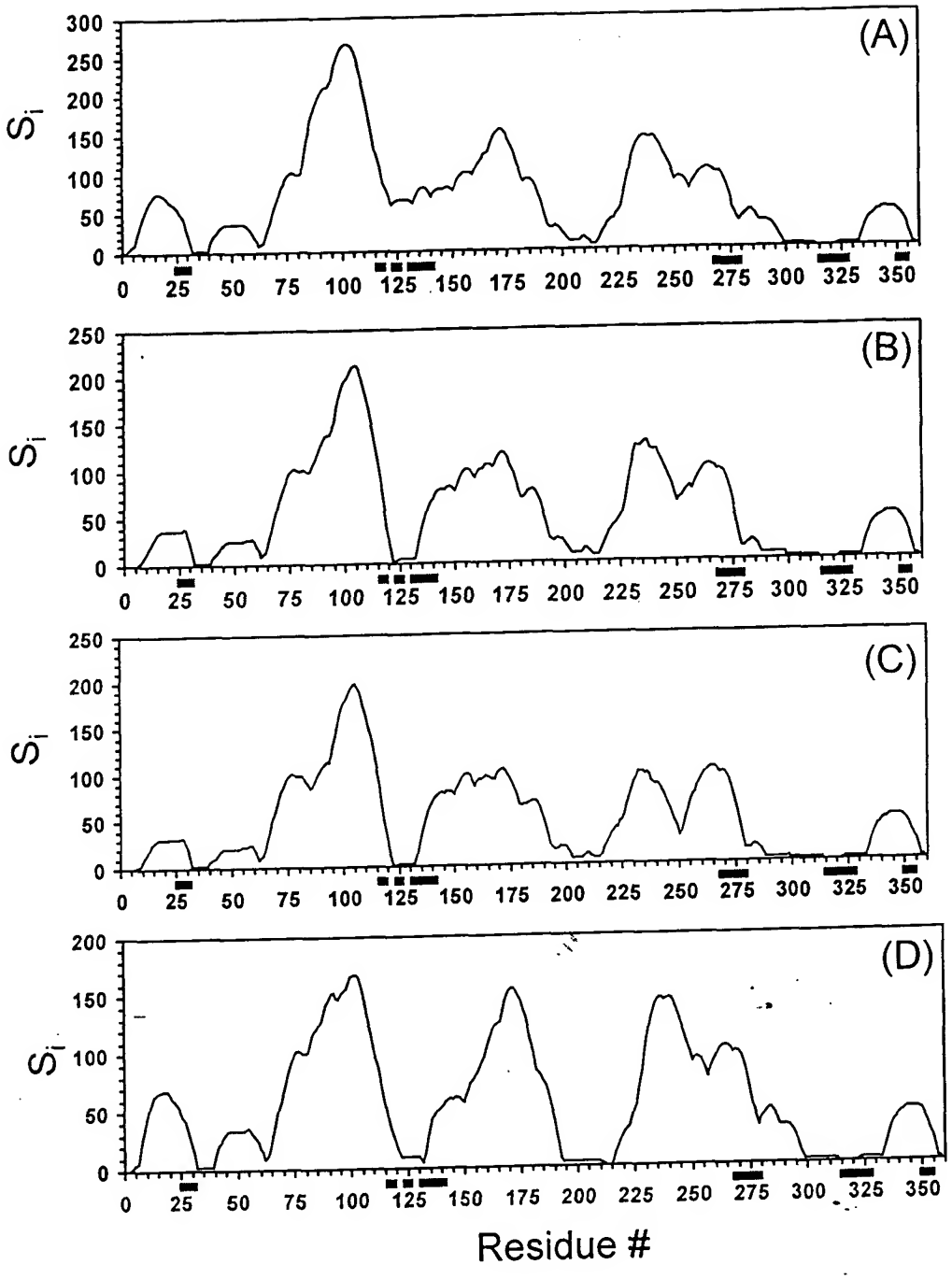


FIG. 25

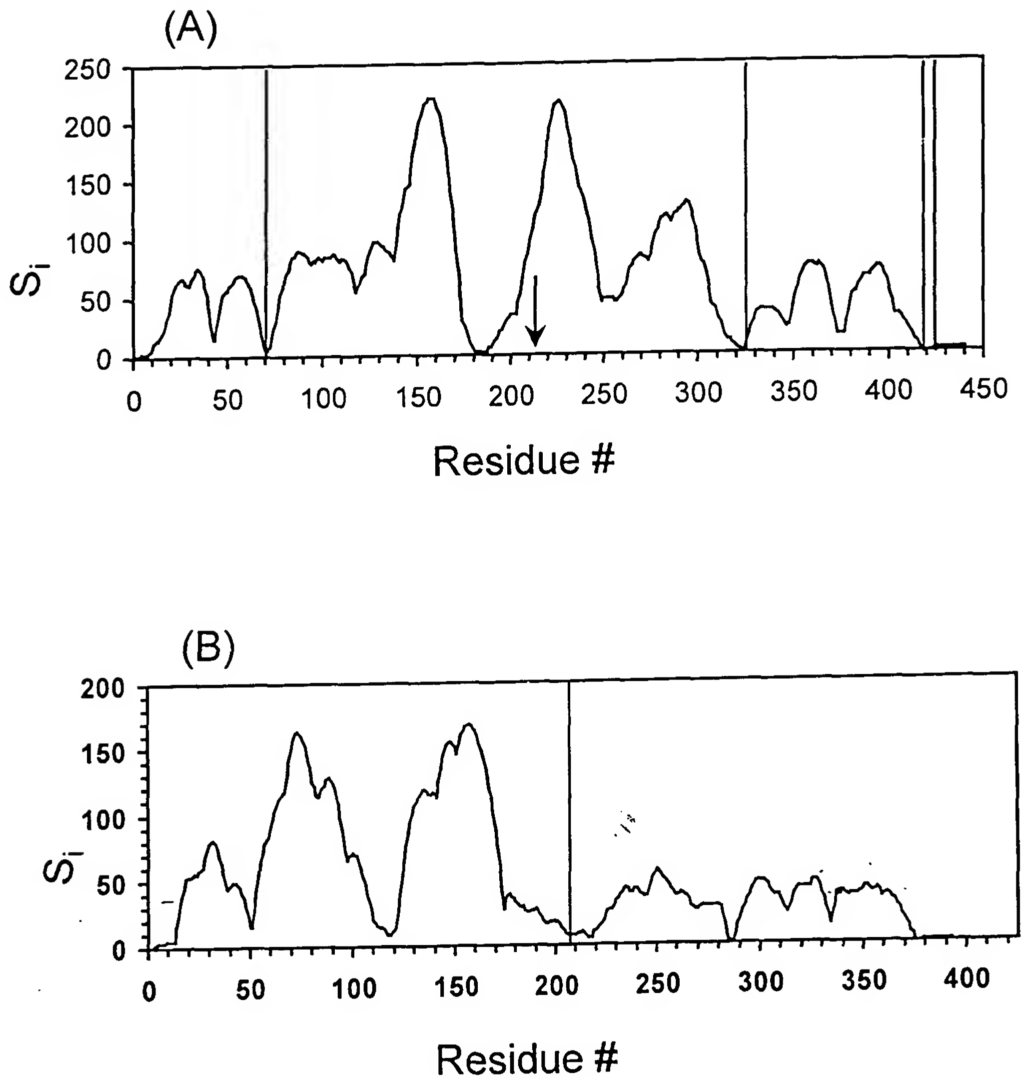


FIG. 26

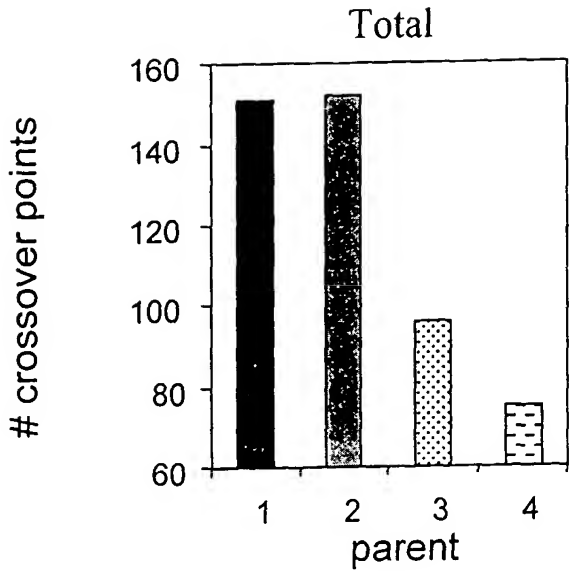


FIG. 27A

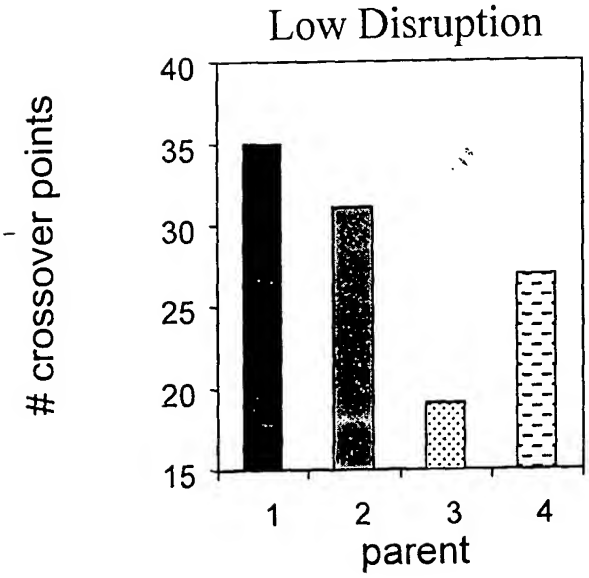


FIG. 27B

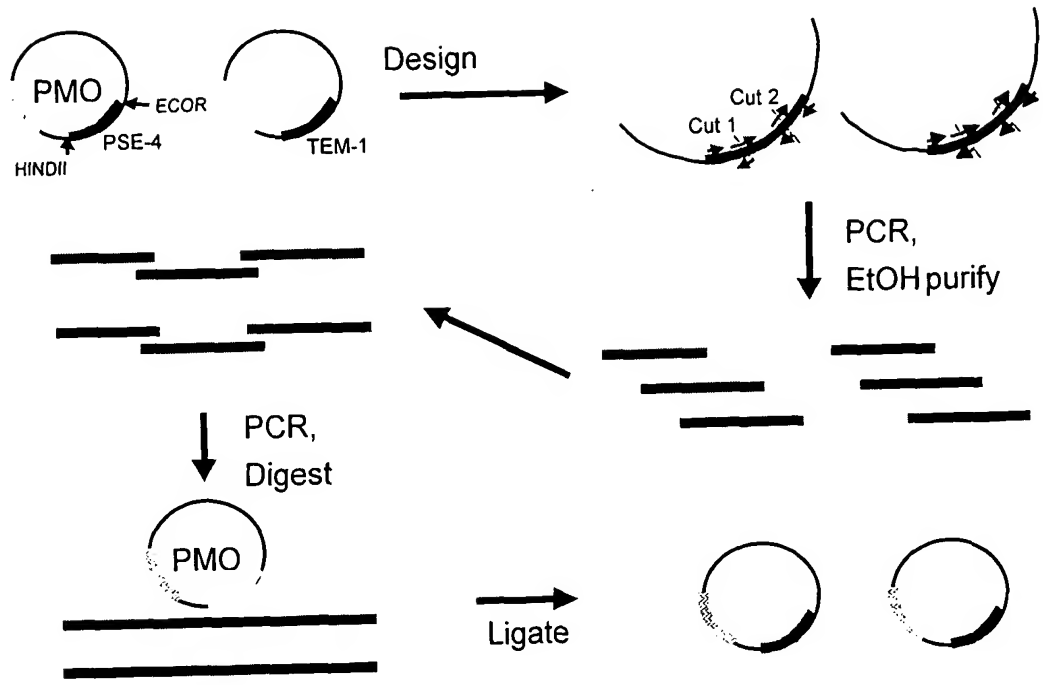

PMO
ECOR
PSE-4
HINDII
TEM-1
Design
Cut 2
Cut 1
PCR,
EtOH purify
PCR,
Digest
PMO
Ligate

FIG. 28

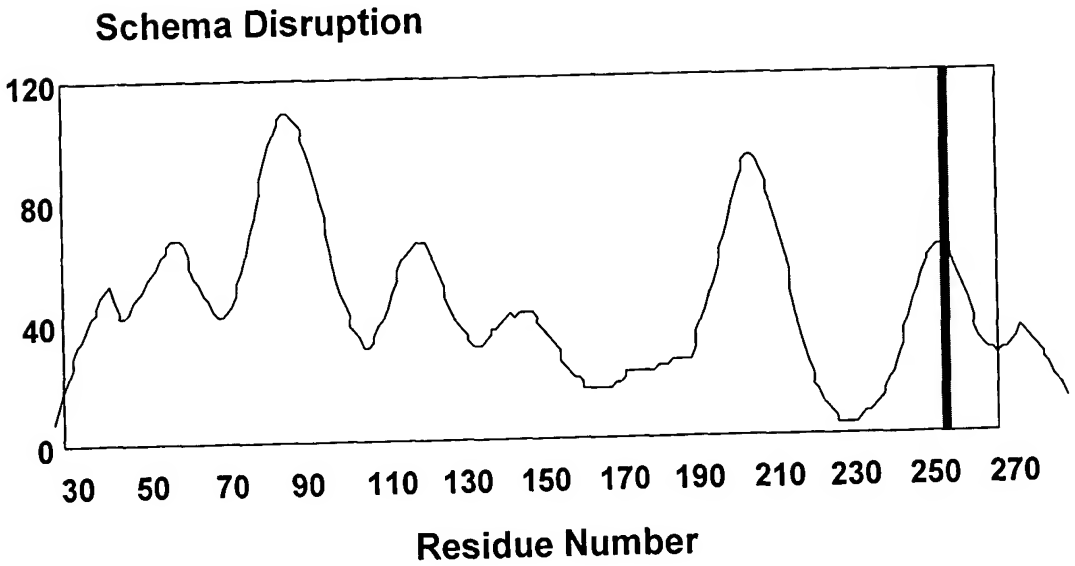

Schema Disruption
120
80
40
0
30
50
70
90
110
130
150
170
190
210
230
250
270
Residue Number

FIG. 29

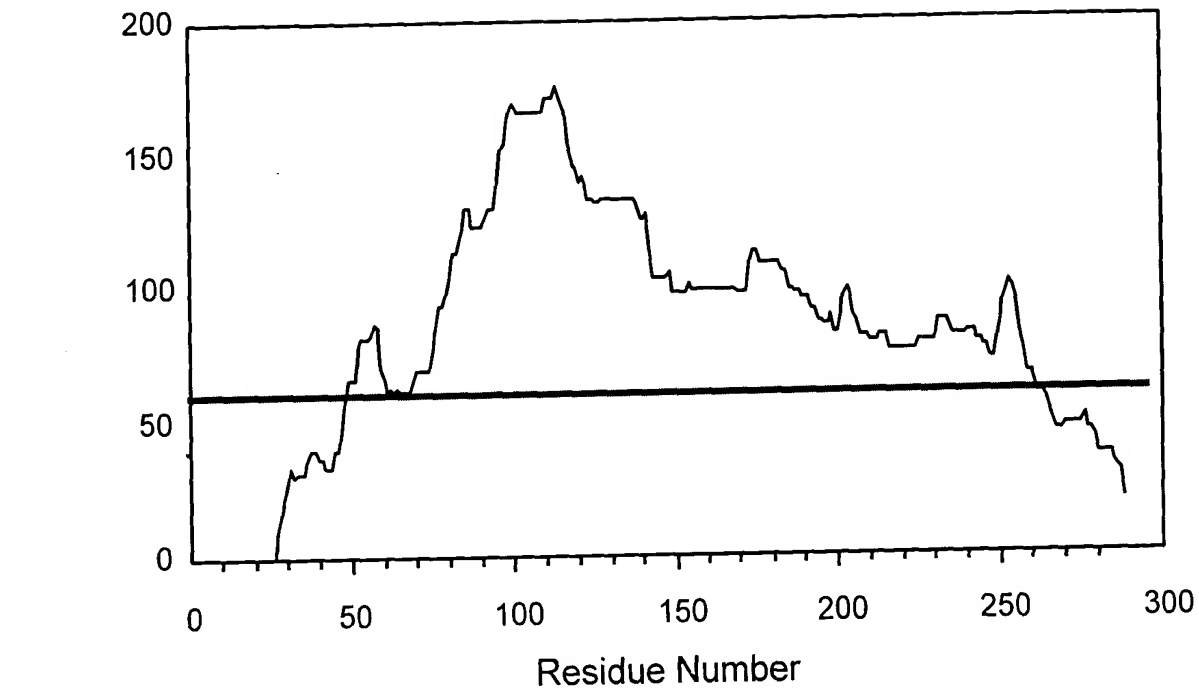

Schema Disruption
200
150
100
50
0
0
50
100
150
200
250
300
Residue Number

FIG. 30

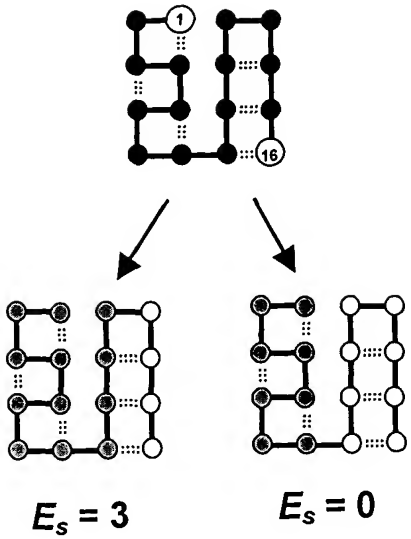


FIG. 31A

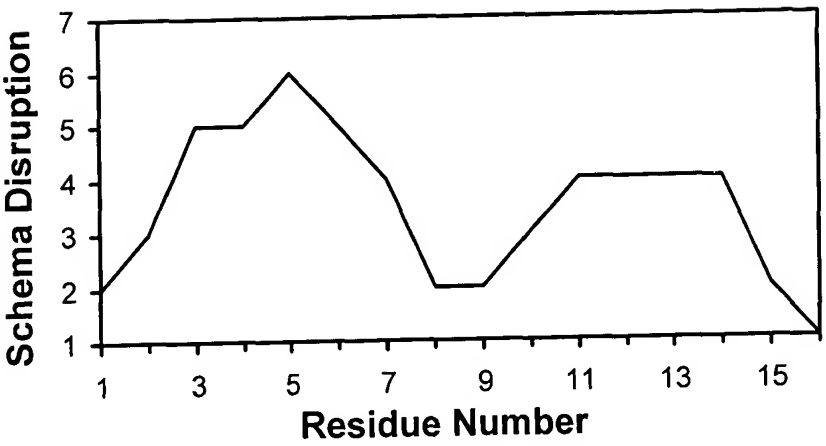


FIG. 31B

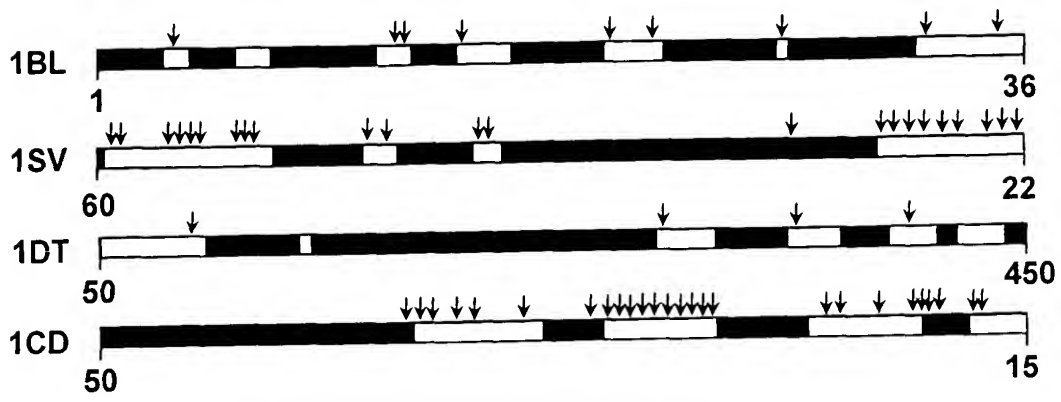

1BL
1
36
1SV
60
22
1DT
50
450
1CD
50
15

FIG. 32

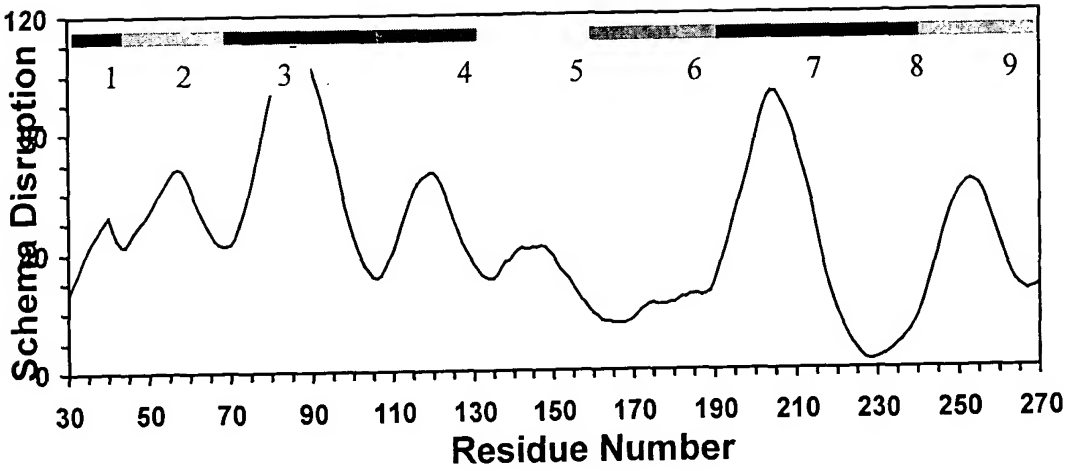

120
Schema Disruption
0
1
2
3
4
5
6
7
8
9
30
50
70
90
110
130
150
170
190
210
230
250
270
Residue Number

FIG. 33

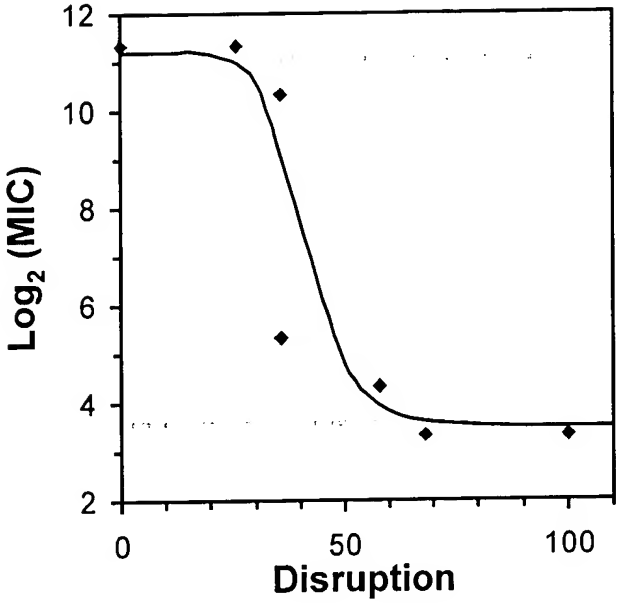


FIG. 34